J. Microbiol. Biotechnol. (2010), **20**(12), 1724–1734
doi: 10.4014/jmb.1007.07030
First published online 6 October 2010

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Cold-Adapted and Rhizosphere-Competent Strain of *Rahnella* sp. with Broad-Spectrum Plant Growth-Promotion Potential

Vyas, Pratibha[1], Robin Joshi[2], K. C. Sharma[3], Praveen Rahi[1], Ashu Gulati[2], and Arvind Gulati[1]*

[1]*Plant Pathology and Microbiology Laboratory, Hill Area Tea Science, Institute of Himalayan Bioresource Technology, Council of Scientific and Industrial Research, Palampur-176 061, Himachal Pradesh, India*
[2]*Biochemistry Laboratory, Hill Area Tea Science, Institute of Himalayan Bioresource Technology, Council of Scientific and Industrial Research, Palampur-176 061, Himachal Pradesh, India*
[3]*Krishi Vigyan Kendra, CSK Himachal Pradesh Krishi Vishvavidyalaya, Bajaura-175 125, Himachal Pradesh, India*

Received: July 14, 2010 / Revised: September 4, 2010 / Accepted: September 14, 2010

A phosphate-solubilizing bacterial strain isolated from *Hippophae rhamnoides* rhizosphere was identified as *Rahnella* sp. based on its phenotypic features and 16S rRNA gene sequence. The bacterial strain showed the growth characteristics of a cold-adapted psychrotroph, with the multiple plant growth-promoting traits of inorganic and organic phosphate solubilization, 1-aminocyclopropane-1-carboxylate-deaminase activity, ammonia generation, and siderophore production. The strain also produced indole-3-acetic acid, indole-3-acetaldehyde, indole-3-acetamide, indole-3-acetonitrile, indole-3-lactic acid, and indole-3-pyruvic acid in tryptophan-supplemented nutrient broth. Gluconic, citric and isocitric acids were the major organic acids detected during tricalcium phosphate solubilization. A rifampicin-resistant mutant of the strain exhibited high rhizosphere competence without disturbance to the resident microbial populations in pea rhizosphere. Seed bacterization with a charcoal-based inoculum significantly increased growth in barley, chickpea, pea, and maize under the controlled environment. Microplot testing of the inoculum at two different locations in pea also showed significant increase in growth and yield. The attributes of cold-tolerance, high rhizosphere competence, and broad-spectrum plant growth-promoting activity exhibited the potential of *Rahnella* sp. BIHB 783 for increasing agriculture productivity.

Keywords: ACC-deaminase activity, indole derivatives, phosphate solubilization, organic acids, plant growth promotion, *Rahnella* sp., rhizosphere competence

Phosphorus is one of the major essential macronutrients often limiting the plant growth because of its low availability in the soil [24, 38]. A large portion of the soluble phosphate fertilizers applied to overcome P deficiency is rapidly rendered unavailable for uptake by the plants, owing to fixation with calcium carbonate, aluminium and iron oxides, and crystalline and amorphous aluminium silicates in the soil [14, 24]. Consequently, the large quantities of phosphatic fertilizers applied to optimize crop productivity impact the energy, environmental, and economic problems. Phosphate-solubilizing soil bacteria have been reported for enhancing growth and yield in several crops, particularly under conditions of poor P availability [14, 45]. The production of phytohormones and antifungal metabolites has also been considered the important mechanisms of plant growth promotion by rhizobacteria. The beneficial influence on plant growth eventually depends on the ability of microbial inoculants to compete with the resident microflora in colonizing the rhizosphere [29]. The cold-adapted and rhizosphere-competent phosphate-solubilizing bacteria with multiple plant growth-promoting attributes could be highly effective as the microbial inoculants in agriculture in colder regions. The present study reports the isolation, characterization, and potential for plant growth promotion of cold-adapted and rhizosphere-competent *Rahnella* sp. strain BIHB 783 from the cold deserts of the Indian trans-Himalayas. Information about cold-adapted strains with plant growth-promoting potential is limited to the strains of genus *Exiguobacterium*, *Pseudomonas*, *Pantoea*, and *Serratia* [30, 32, 43, 44, 45, 46]. The plant growth-promoting potential of the genus *Rahnella* has been studied for acetylene reduction in pure culture and in association with wheat and maize, and solubilization of mineral phosphates by *Rahnella aquatilis* [5, 25, 27], growth promotion by *R. aquatilis* in Indian mustard [28], possible role of *R. aquatilis* in protecting chardonnay vine against galling by *Agrobacterium vitis* [4], and reduced susceptibility in tomato seedlings pretreated with *R. aquatilis* strains antagonistic to *Xanthomonas campestris*

*Corresponding author
Phone: +911894230411, 911894230454; Fax: +911894230433;
E-mail: gal_arvind@yahoo.co.in, arvindgulati@ihbt.res.in

pv. *vesicatoria* [12]. Enhanced phytoextraction of Ni and Cd from fly ash has also been reported by Indian mustard inoculated with *R. aquatilis* [28].

Materials and Methods

Soil Sampling and Isolation of Bacteria

Several phosphate-solubilizing bacteria were isolated from the soil samples collected from the rhizosphere of *Hippophae rhamnoides* growing at Rong Tong (32° 16' 07" N and 78° 04' 22" E; 3,900 m amsl) in Lahaul and Spiti in the Indian trans-Himalayan region. Serial soil dilutions were spread-plated on modified Pikovskaya agar, and the bacterial colonies producing a distinct tricalcium phosphate (TCP) solubilization zone were grown in pure culture and maintained in 20% glycerol at −70°C. A bacterial isolate BIHB 783 producing a sharp phosphate-solubilization zone of about 22 mm breadth after 5 days of incubation and morphologically different from other isolates, was selected for further studies. The culture has been deposited under Accession No. MTCC 5338 at the Microbial Type Culture Collection and GenBank.

Characterization of the Bacterial Isolate

Phenotypic characterization. Phenotypic characterization of the isolate BIHB 783 was done based on colony morphology, growth characteristics, microscopic observations, and biochemical tests [26].

Carbon source utilization. The carbon-source utilization pattern for 95 carbon sources by the bacterial isolate was studied using BIOLOG system (BIOLOG MicroStation). The culture grown in triplicate on TSA for 48 h at 30±0.1°C was resuspended in 20 ml of inoculation fluid (BIOLOG) with a sterile cotton swab and the inoculum density was adjusted to 52% transmittance using the BIOLOG Turbidimeter. BIOLOG GN microplates were inoculated with 150 µl of the adjusted bacterial suspension per well and incubated at 30°C for 24 h. The development of colour was read at 595 nm filter in the MicroStation Reader between 4–6 h and 16–24 h of incubation. The substrate-utilization profiles were compared with the MicroLog software version 4.2 database and the identification acknowledged when the similarity index was ≥0.5.

Whole-cell fatty acid methyl ester analysis. The whole-cell fatty acids of the bacterial isolate derivatized to methyl esters in triplicate were analyzed by gas chromatography (GC) using the Sherlock Microbial Identification System (MIDI, Inc., Newark, DE, USA) as described earlier [41]. The fatty acids from the bacterial culture grown on trypticase soya agar for 24 h at 28°C were extracted by saponification in dilute sodium hydroxide and methanol solution, followed by derivatization with dilute hydrochloric acid and methanol solution to give methyl esters, and extracted with an organic solvent consisting of hexane and methyl *tert*-butyl ether. The resulting extract was analyzed by a GC system (GC 6890; Agilent Technologies, USA) using an Ultra 2 phenyl methyl silicone fused silica capillary column of 25 m×0.2 mm (Agilent Technologies, USA), with hydrogen as the carrier gas, nitrogen as the "make up" gas, and air to support the flame. The GC oven temperature was programmed from 170 to 270°C at 5°C rise per minute with 2 min hold at 300°C. The fatty acids were identified and quantified by comparison with the retention times and peak areas obtained for the authentic standards. Qualitative and quantitative differences in the fatty acid profiles were used to compute the distance for the strain relative to the strains in the Sherlock bacterial fatty acid reference libraries TSBA50 5.00.

16S rRNA gene sequencing. Genomic DNA of the bacterial isolate was extracted using a Qiagen DNeasy Plant Mini kit (Qiagen). Amplification of 16S rDNA was performed using fD1 (5'-AGAGT TTGATCCTGGCTCAG-3') and rP2 (3'-ACGGCTACCTTGTTACG ACTT-5') primers [53]. The total volume of PCR reaction mixture was 50 µl, comprising 200 µM dNTPs, 50 µM of each primer, 1× PCR buffer, 3U *Taq* DNA polymerase, and 100 ng genomic DNA. The thermocycling procedure involved an initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min, and final extension at 72°C for 8 min. The gel-purified 16S rDNA was ligated to pGEM-T easy vector (Promega) and transformed in *E. coli* JM109. The sequence of the insert was determined using Big-Dye Terminator Cycle Sequencing and ABI Prism 310 Genetic Analyzer. The sequence was analyzed using the gapped BLASTn (http://www.ncbi.nlm.nih.gov) search algorithm and aligned to their nearest neighbors. The evolutionary distance of the isolate and its related taxa was calculated with TREECON software package version 1.3b (Yves Van de Peer, University of Antwerp, 1994, 1997) using Kimura's two-parameter model after aligning the sequences with ClustalW. The 16S rRNA gene sequence of *Pseudomonas putida* strain ATCC 12633 was used as the outgroup. The gene sequence has been submitted under Accession No. DQ885948 with the NCBI GenBank database.

Growth at Different Temperatures

Growth of the bacterial isolate was studied at different temperatures in comparison with a mesophilic strain *E. coli* DH5α in nutrient broth. Fifty ml of nutrient broth in triplicate was inoculated with 500 µl of 24-h-old bacterial cultures and the OD_{600} adjusted to 0.02 for both strains with sterile nutrient broth. The flasks were incubated at 10, 20, 30, and 37°C in an incubator shaker for 72 h. Culture (1 ml) was sampled every 2 h, centrifuged at 10,000 rpm for 10 min, the pellet suspended in 1 ml of normal saline, and the absorbance read at 600 nm against a normal saline blank.

Screening for Plant Growth-Promoting Attributes

Phosphate solubilization. The test strain was grown, in triplicate, in 50 ml of National Botanical Research Institute's Phosphate (NBRIP) broth [31] containing 0.5% tricalcium phosphate (TCP) and incubated at 28°C for 5 days at 180 rpm in a refrigerated incubator shaker (Innova Model 4230; New Brunswick Scientific, USA). Cultures were centrifuged at 10,000 rpm for 10 min and passed through a 0.22-µm nylon filter, and the phosphorus content in the culture filtrates was estimated by the vanadomolybdate method [22]. The uninoculated autoclaved media with TCP as phosphate substrate, used as the control, were incubated under a similar set of conditions as the inoculated cultures. Values of P liberated are expressed as µg/ml over the control.

Organic acid production during phosphate solubilization. High-performance liquid chromatography (HPLC) analysis of the culture filtrates, in triplicates, obtained on solubilization of TCP was done on a Waters 996 HPLC equipped with a PDA detector, Waters 717 plus auto-sampler, Waters 600 controller, WatersTM pump, Waters inline degasser AF, and Lichrosphere RP-18 column (250 mm× 4.6 mm and 5 µm particle size; Merck, Germany). The mobile phase was 0.1% orthophosphoric acid (Merck, Germany) in a gradient

programme of flow rate starting at 0.4 ml/min at 0 min, increasing to 0.6 ml/min at 9.5 min, decreasing from 0.6 to 0.4 ml/min from 9.5 to 18 min, isocratic at 0.4 ml/min from 18 to 25 min, again increasing from 0.4 to 0.6 ml/min from 25 to 30 min, and equilibrating to 0.4 ml/min from 30 to 35 min. The eluates were detected at λ 210 nm and identified by retention time and co-chromatography of the samples spiked with the authentic standards of organic acids [gluconic acid (Sigma-Aldrich, USA), 2-keto gluconic acid (Sigma, USA), formic acid, isocitric acid, lactic acid, oxalic acid, succinic acid, maleic acid, citric acid, and fumaric acid (Supelco, USA)]. Each replicate was analyzed in a single run on HPLC and the values were presented as the mean of three replicates.

Phytase activity. Production of phytases by the isolate was determined on phytate screening agar medium with 0.5% calcium phytate after 5 days of incubation at 28°C [39].

Production of IAA-like auxins. The bacterium was grown in nutrient broth supplemented with 0.1% DL-tryptophan for 48 h at 180 rpm. Colorimetric estimation of IAA-like auxins was first done using Salkowski reagent [40]. Quantification of indole derivatives in the ethyl acetate extract of the culture supernatant was done on a Waters 996 HPLC, equipped with a PDA detector, Waters 717 plus auto sampler, Waters 600 controller, and Lichrosphere RP-18 column 250 mm×4.6 mm and 5 µm particle size (Merck, Germany), as described earlier [8]. A gradient mobile phase of 0.5% acetic acid (A) and 100% acetonitrile (B) with 60% A and 40% B for 0–6.5 min, 55% A and 45% B for 6.5–8.0 min, and 60% A and 40% B for 8.0–12.0 min was employed at a flow rate of 1 ml/min. Eluates were detected using an isocratic system and identified by retention time and co-chromatography by spiking the sample with authentic standards [tryptophan (try), indole-3-acetic acid (IAA), indole-3-pyruvic acid (IPA), indole-3-acetaldehyde (IAAld), indole-3-acetamide (IAM), and indole-3-acetonitrile (IAN)]. The samples were analyzed in triplicate.

1-Aminocyclopropane-1-carboxylate (ACC) deaminase activity. ACC-deaminase activity was initially detected on plates with DF minimal medium containing ACC as nitrogen source [21]. The germinating seed bioassay for ethylene reduction due to ACC deaminase activity was performed by measuring the root length of surface-sterilized pregerminated seeds of *Pisum sativum* var. Palam Priya and *Zea mays* var. Girija treated for 1 h with 48-h-old bacterial culture (OD_{600}=1.0~1×10^9 CFU/ml) in NB, as compared with uninoculated controls after 5 days of incubation at 28°C in Petri plates [9]. The enzyme activity in the culture filtrates was determined by measuring the production of α-ketobutyrate generated by ACC cleavage by ACC deaminase using 2,4-dinitrophenylhydrazine reagent [36]. Nutrient broth (15 ml) inoculated with 100 µl of 48-h-old bacterial culture was incubated at 28°C for 48 h; the cultures were then centrifuged for 10 min at 4,000 rpm and 4°C, and the pellets suspended in 5 ml of DF salts minimal medium and centrifuged again at 3,000 rpm at 4°C for 5 min. The supernatant was discarded and the pellets were suspended in 7.5 ml of DF minimal medium supplemented with 3.0 mM final concentration of ACC and incubated at 28°C. After 24 h, the cultures were centrifuged at 10,000 rpm for 10 min at 4°C and the pellets washed twice with 0.1 M Tris-HCl, pH 7.5, and suspended in 600 µl of 0.1 M Tris-HCl, pH 8.5. Bacterial cells made labile by adding 30 µl of toluene were vortexed at the highest speed for 30 s. A 100-µl aliquot of the toluenized cells was stored at 4°C for protein estimation. The remaining toluenized cell suspension was assayed for ACC deaminase activity. To a 200-µl aliquot of the cell suspension, 20 µl of 0.5 M ACC was added, followed by vortexing for 30 s and incubating at 30°C for 30 min. The contents were transferred to a 10-ml glass vial, 1 ml of 0.56 N HCl was added and centrifuged for 5 min at 13,000 rpm at 4°C. One ml of supernatant was vortexed with 800 µl of 0.56 N HCl; thereafter, 300 µl of 2,4-dinitrophenylhydrazine reagent was added. The contents were vortexed and incubated at 30°C for 30 min. Following the addition of 2 ml of 2 N NaOH, the absorbance was measured at 540 nm in an Ultraspec 3000 UV/Visible Spectrophotometer. The negative control for the assay included 200 µl of labilized cell suspension without ACC and the blank included 200 µl of 0.1 M Tris-HCl (pH 8.5) with 20 µl of 0.5 M ACC. The protein concentration of the toluenized cells was determined using a bicinchoninic acid protein assay kit (Novagen, Merck, Germany) according to the manufacturer's instructions. The ACC deaminase activity was expressed as nM α-ketobutyrate/mg protein/h.

Siderophore production. Siderophore production was determined by performing the universal chemical assay in CAS agar plates [42]. Quantitative estimation of siderophores was done in the cell-free extract of 5-day-old cultures grown at 28°C in MM9 medium by CAS-shuttle assay [35].

Ammonia production. Accumulation of ammonia was detected by adding Nessler's Reagent to a cell-free extract of 48-h-old bacteria grown in peptone water [6].

Hydrogen cyanide (HCN) production. HCN production by the strain was determined on TSA plates supplemented with glycine after 3 days of incubation at 28°C [7].

Inoculum Preparation

The bacterium grown in 20 ml of trypticase soya broth (TSB) for 48 h at 28°C was centrifuged at 10,000 rpm for 10 min and the pellet suspended in 0.85% NaCl (OD_{600}=1.0). The bacterial suspension was mixed with sterilized activated charcoal [4:6 (v/w)] to 10^7 CFU/g.

Evaluation for Plant Growth Promotion under Controlled Environment

Seeds of *Pisum sativum* var. Palam Priya, *Zea mays* var. Girija, *Hordeum vulgare* var. Dolma, and *Cicer arietinum* var. HPG 17 were surface-sterilized with 20% NaOCl for 3 min, washed three times with sterile distilled water, and germinated at 25°C in moist sterile vermiculite. The uniformly germinated seeds coated with the slurry of charcoal-based bacterial inoculant were sown in 15-cm diameter pots filled with sterilized vermiculite. The pregerminated seeds treated with sterilized activated charcoal without bacterial inoculant served as the control. The pots were placed in a completely randomized block design, with eight replications, under a mixed incandescent and fluorescent illumination of 550 µM photon/m^2/s with 16/8 h light/dark cycle at 25±2°C and 50–60% RH in an Environment Control Chamber. Hoagland nutrient solution diluted 10-fold was given at 10-day intervals to the plants. The data on root length, shoot length, and total dry weight were recorded after 30 days of sowing. The plants were oven-dried at 70°C for 3 days to determine the total dry weight.

Field Evaluation

Field testing of the inoculant in comparison with untreated control was done on *Pisum sativum* var. Palam Priya from November–February 2007, in microplots measuring 1 m^2 with three replicates

for both the treatments at the Institute of Himalayan Bioresource Technology Chandpur Experimental Farms, Palampur (32° 6' 0" N and 76° 31' 0" E; 1,300 m amsl) and Hill Area Research and Extension Centre (HAREC) CSK HPKV Experimental Farms, Bajaura (31° 50' 19" N and 77° 10' 23" E; 1,090 m amsl). The seeds treated with the inoculum as described above were sown at an inter-plant distance of 3 cm and inter-row distance of 25 cm. Data on plant growth, dry weight, and yield in terms of pod fresh weight were recorded after 110 days of sowing on randomly selected nine plants from each replicate.

Rifampicin Tagging

The spontaneous rifampicin-resistant mutants of the wild strain BIHB 783 were generated by transferring bacterial culture from TSA to TSB containing increasing concentrations of rifampicin (25, 50, 100 µg/ml) and plating a loopful of the culture on TSA plates amended with 100 µg rifampicin/ml (TSA-rif). The spontaneous rifampicin-resistant mutant (BIHB 783^{Rif}) showing growth comparable with the wild strain (BIHB 783) on TSA-rif plates was selected and checked for stability by transferring four times from TSA-rif to TSA and TSA-rif plates.

Root Colonization and Influence of Rifampicin-Resistant Mutant on Soil Microflora and Plant Growth

The surface-sterilized pregerminated *Pisum sativum* var. Palam Priya seeds treated with charcoal-based BIHB 783Rif inoculant and sterilized activated charcoal (control) were sown in 15-cm diameter pots filled with nonsterilized soil in 24 replications in an Environment Control Chamber. Initial count of BIHB 783^{Rif} per seed was determined by plating the serial dilutions on TSA-rif plates. Total viable count of bacteria, actinomycetes, fungi, and BIHB 783^{Rif} in root homogenates and non-rhizosphere soils were determined at weekly intervals for eight weeks, by plating serial dilutions on TSA, YME, SDA, and TSA-rif plates, respectively. The plates prepared in triplicate were incubated at 28°C for 48 h for bacteria and for 72 h for actinomycetes and fungi.

Data were recorded on root length, shoot length, and total dry weight after 60 days of sowing to compare the mutant strain with the wild strain for influence on plant growth by applying charcoal-based inoculum as described above to surface-sterilized pregerminated seeds in nonsterilized soil.

Experimental Design and Data Analysis

A randomized block design was adopted for conducting the experiments. The data were checked for normality and subjected to analysis of variance (ANOVA) using the STATISTICA data analysis software system version 7 (StatSoft Inc., Tulsa, USA). All values are means of three replicates and experiments were repeated thrice unless stated otherwise. The mean of treatments was compared by CD value at P=0.5 and 0.01.

Results and Discussion

Isolation and Characterization of the Bacterial Isolate

The bacterial strain morphologically different from other colonies, producing a sharp and prominent (about 22 mm) zone of phosphate solubilization after 5 days of incubation on modified Pikovskaya agar, showed resemblance to *Rahnella* for the major phenotypic characteristics [26]. The bacterial colonies were circular, smooth, raised, and entire. The bacterial strain was Gram-negative, motile rods; positive for catalase, citrate utilization, starch hydrolysis, esculin hydrolysis, Voges–Proskauer test, and urease; and negative for indole, oxidase, gelatin hydrolysis, and methyl red test.

The bacterial isolate showed utilization of dextrin, tween 40, tween 80, *N*-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, β-methyl D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, methyl pyruvate, monomethyl succinate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, α-ketoglutaric acid, D,L-lactic acid, malonic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, glucuronamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-histidine, L-proline, L-serine, inosine, uridine, thymidine, glycerol, D,L-α-glycerol phosphate, and glucose-1-phosphate. The strain showed the maximum similarity index of 0.32 with *Rahnella aquatilis* in the BIOLOG database. However, the isolate differed from *R. aquatilis* in utilizing *cis*-aconitic acid, malonic acid, glucuronamide, D-alanine, L-glutamic acid, L-histidine, and L-proline, and not utilizing adonitol, D-arabitol, and glucose-6-phosphate as the carbon source. The cell wall fatty acid composition showed 12:0, 14:0, 16:1 w7c/16:1 w6c, 14:0 3OH/16:1 iso I, 16:0, 17:0 cyclo, and 18:1 w7c as the predominant forms of fatty acids, along with small amounts of 12:0 3OH, 13:0, 16:0 3OH, 16:1 w5c, 17:0, 17:0 iso, 17:1 w7c, 18:0, 18:1 2OH, 18:1 w5c, 18:2 w6,9c/18:0 ante, 18:1 w7c 11-methyl, 19:0, 19:0 cyclo w8c, 19:0 cyclo w10c/19w6, 19:1 w6c/w7c/19cy 20:0, and 20:1 w7c. The molecular analysis based on 16S rRNA gene homology of the 1,499 bp partial sequence identified the test strain as *Rahnella* sp. The phylogenetic analysis grouped the strain with *Rahnella aquatica* DSM 4594, *R. aquatilis* U90757, and *R. aquatilis* ATCC 33989 at 99% similarity (Fig. 1).

Growth at Different Temperatures

Rahnella sp. BIHB 783 showed growth in the temperature range of 10–37°C as compared with the growth over 20–37°C of the mesophilic control strain *E. coli* DH5α (Fig. 2). The growth at 20 and 30°C was significantly higher with the shorter lag phase in the test strain than the mesophilic strain. At 37°C growth of the test strain was slower, attaining the stationary phase at 20 h as compared with the mesophilic strain, which reached the stationary phase at 8 h of incubation. The results revealed that *Rahnella* sp. BIHB 783 is a cold-adapted psychrotrophic strain. The selection

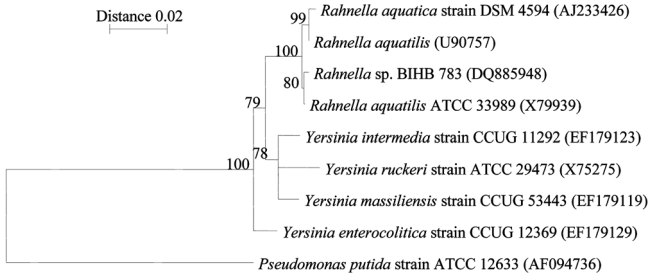

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences using the neighbor-joining method to draw the relationship among *Rahnella* sp. strain BIHB 783 and closely related genera. Numbers on nodes indicate bootstrap values. 16S rRNA gene accession numbers are given within brackets. Bar=0.02 substitutions per site.

of microorganisms for cold tolerance is an important parameter in developing effective plant growth-promoting microbial inoculants for low-temperature regions. Earlier reports on the selection of cold-tolerant plant growth-promoting bacteria are limited to *Exiguobacterium acetylicum*, *Pantoea dispersa*, *Pseudomonas fluorescens*, *Pseudomonas fragi*, and *Pseudomonas* sp. from the North-western Indian Himalayas [30, 32, 43, 44, 45, 46].

Screening for Plant Growth-Promoting Activities

The results on screening of *Rahnella* sp. strain BIHB 783 for various plant growth-promoting attributes are given in Table 1. The test strain exhibited TCP solubilization comparable to *Acinetobacter rhizosphaerae*, *Pantoea agglomerans*, *Pseudomonas trivialis*, *P. poae*, *P. fluorescens* and *Pseudomonas* spp. reported for high phosphate solubilization [17, 18, 23, 47]. The native strains with high TCP solubilization could prove highly beneficial to plant growth in calcium-rich and alkaline soils where binding of P with Ca creates deficiency in the availability of phosphorus [18, 51]. The production of organic acids and their chelation capacity have been implicated as the major

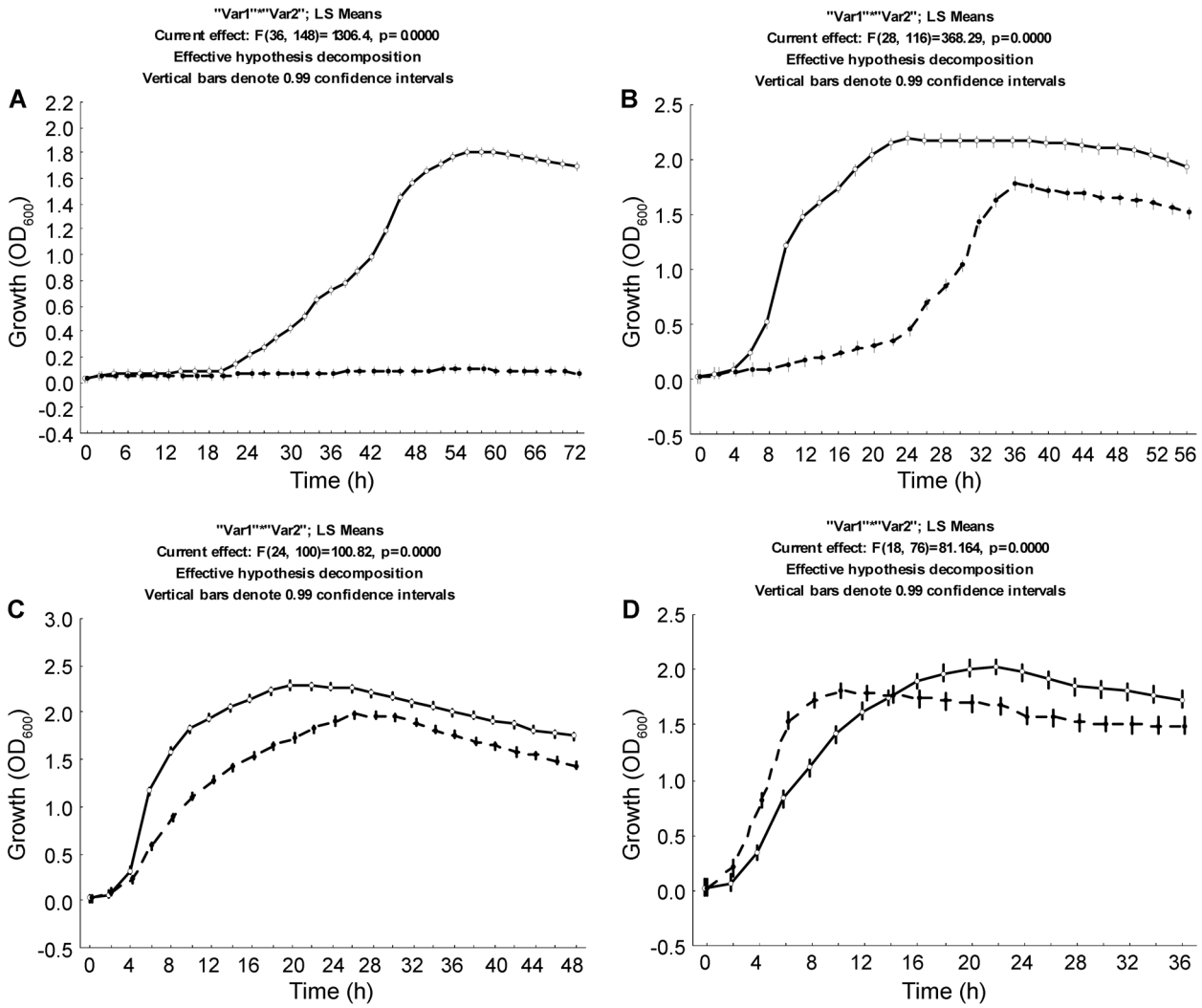

Fig. 2. Growth of *Rahnella* sp. BIHB 783 (solid lines) and *E. coli* DH5α (dotted line) at 10°C (**A**), 20°C (**B**), 30°C (**C**), and 37°C (**D**).

Table 1. Plant growth-promoting attributes of *Rahnella* sp. strain BIHB 783.

PGP attribute		Activity
TCP solubilization (μg/ml)		805.00±6.40
Organic acid production (μg/ml):		
Gluconic acid		235.2±8.13
Isocitric acid		140.7±6.43
Citric acid		124.6±4.75
2-Ketogluconic acid		28.5±0.56
Oxalic acid		0.32±0.01
Fumaric acid		0.14±0.02
Phytate solubilization zone (mm)		5.20±0.80
Indole derivatives:		
Colorimetric (μg/ml)		24.50±1.50
HPLC (μg/ml)	IAA	2.45±0.09
	IAAld	0.54±0.06
	IAN	0.36±0.07
	IAM	2.77±0.09
	ILA	13.3±0.42
	IPA	8.67±0.31
ACC deaminase activity:		
Germinating seed bioassay (% root elongation):		
Zea mays var. Girija		61*
Pisum sativum var. Palam Priya		53*
Enzyme activity (nM α-ketobutyrate/h mg protein)		3288.5±9.50
Siderophore production:		
Zone (mm)		27.20±1.04
Units (%)		88.00±3.60
Ammonia production		+ve
HCN production		–ve

Values are the mean of three replicates ± standard deviation, *significantly different from control at $P<0.01$. PGP=plant growth-promoting, TCP=tricalcium phosphate, IAA=indole-3-acetic acid, IPA=indole-3-pyruvic acid, IAM=indole-3-acetamide, ILA=indole-3-lactic acid, IAAld=indole-3-acetaldehyde, Trp=tryptophan, IAN=indole-3-acetonitrile, ACC=1-aminocyclopropane-1-carboxylate and HCN=hydrogen cyanide.

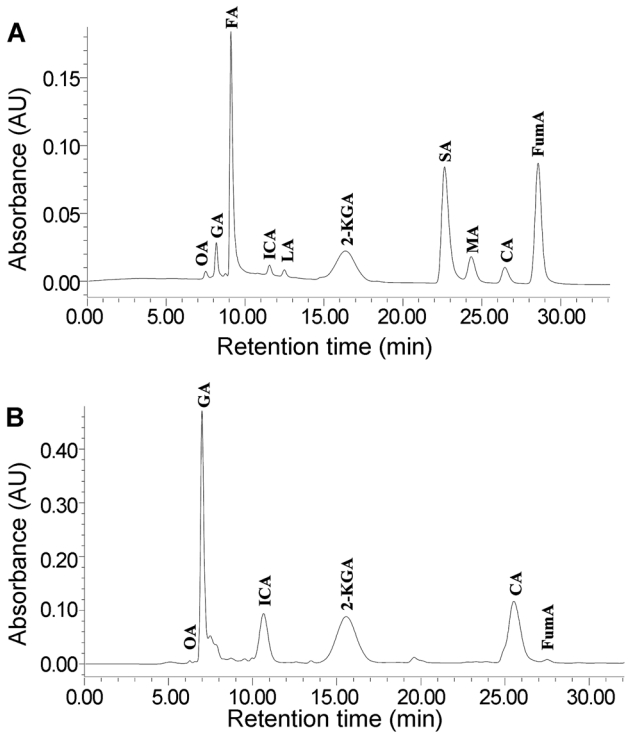

Fig. 3. HPLC chromatograms of authentic organic acids (**A**) and culture supernatant of *Rahnella* sp. BIHB 783 grown for 5 days in National Botanical Research Institute's Phosphate broth (**B**). OA=oxalic acid, GA=gluconic acid, FA=formic acid, ICA=isocitric acid, LA=lactic acid, 2-KGA=2-ketogluconic acid, SA=succinic acid, MA=maleic acid, CA=citric acid, and FumA=fumaric acid. Peaks were identified by co-chromatography by spiking the sample with authentic compounds.

mechanism in the solubilization of inorganic phosphates by microorganisms [33, 34]. Gluconic, citric, and isocitric acids were detected as the major organic acids with small quantities of 2-ketogluconic, oxalic, and fumaric acids during TCP solubilization by the test strain (Fig. 3; Table 1). Gluconic acid has also been reported as the major organic acid during phosphate solubilization by *Azospirillum* spp., *Citrobacter* sp., *Pseudomonas cepacia*, *P. fluorescens*, *P. poae*, *P. trivialis*, and *Rahnella aquatilis* [25, 33, 34, 38, 50]. The strain also showed the ability of organic phosphate solubilization as reported for *Bacillus*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Serratia*, and *Yersinia* by releasing phosphorus from phytic acid, which is a predominant form of organic phosphates in the soil [19, 20, 38].

The present studies showed the production of rooting hormone IAA by *Rahnella* sp. BIHB 783, which is higher than the earlier report of IAA production by *Acinetobacter rhizosphaerae* from the cold deserts of Indian trans-Himalayas [18]. IAA production in the presence of a suitable precursor such as tryptophan has been reported for several other PGPR belonging to *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Pantoea*, *Pseudomonas*, and *Serratia* [18, 48]. The presence of IAA, IAAld, IAM, ILA, IAN, and IPA, which are the intermediaries of IAA biosynthetic pathways, was detected in the culture supernatants (Fig. 4) [8, 15].

Rahnella sp. BIHB 783 showed growth in DF medium with ACC as the sole nitrogen source and also significantly enhanced root length in pea and maize over uninoculated controls, indicating the production of ACC deaminase by the bacterium. The bacteria producing ACC deaminase are known to promote root elongation and increase the active rhizosphere zone by lowering the ethylene levels through hydrolysis of ACC given off by the germinating seeds [16]. The strain exhibited high ACC deaminase activity comparable to the enzyme activity of the efficient PGPR strains including *Burkholderia kururiensis*, *Dyella ginsengisoli*, *Leifsonia shinshuensis*, *Microbacterium phyllosphaerae*,

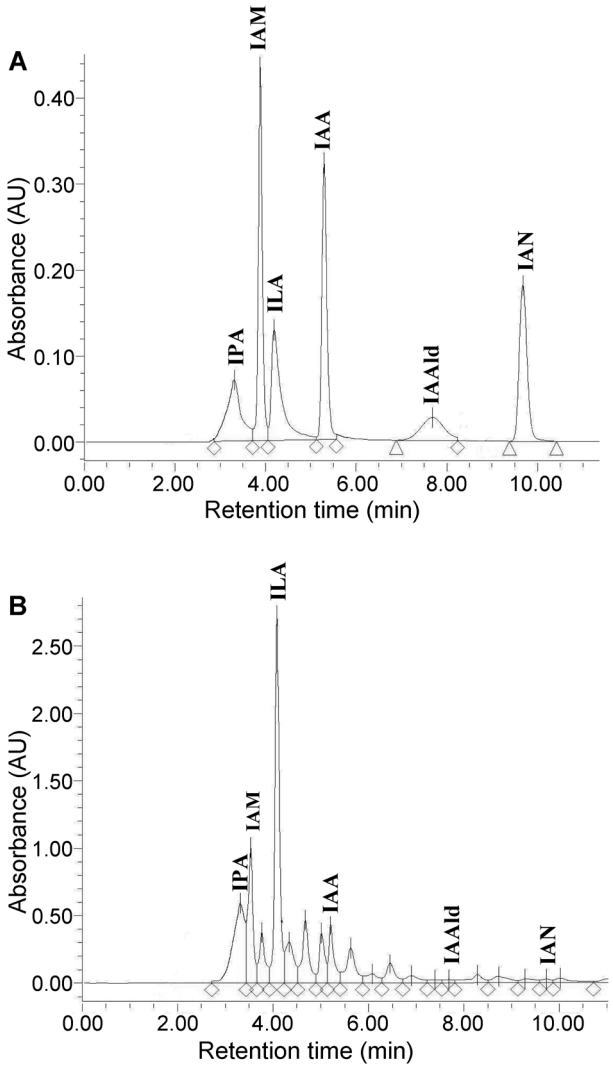

Fig. 4. HPLC chromatograms of authentic indole compounds (**A**) and the ethyl acetate extract of culture supernatant of *Rahnella* sp. BIHB 783 grown in nutrient broth with tryptophan (**B**).
IPA=indole-3-pyruvic acid, IAM=indole-3-acetamide, ILA=indole-3-lactic acid, IAA=indole-3-acetic acid, IAAld=indole-3-acetaldehyde, and IAN=indole-3-acetonitrile. Peaks were identified by co-chromatography by spiking the sample with authentic compounds.

Pandoraea sputorum, *Pseudomonas fluorescens*, and *Pseudomonas putida* [1, 13, 37]. Recently, plant growth-promoting *R. aquatilis* has been reported for ACC deaminase activity [28].

Rahnella sp. BIHB 783 also exhibited the production of siderophore, which is an important trait of PGPR that suppresses the growth of fungal pathogens in the rhizosphere by chelating iron. The test strain could also be indirectly augmenting the availability of P, as the siderophores due to their high affinity for iron, are also involved in the release of iron-bound phosphorus [10]. The strain also exhibited the production of ammonia taken up by plants as a source of nitrogen for growth [9]. The strain tested negative for the production of HCN involved in plant growth reduction [2, 49]. The rhizobacteria with multiple growth-promoting mechanisms have been considered important to enhancing growth and yield in different crops [9, 18].

Table 2. Plant growth promotion in pea by wild and mutant strains of *Rahnella* sp. BIHB 783 in non-sterilized soil after 60 days in Environment Growth Chamber.

Growth Parameter	Control	Wild strain	Rif-resistant mutant	CD at $P<0.01$
Root length (cm)	20.3	24.5	23.4	0.75
Shoot length (cm)	28.4	35.4	34.3	1.21
Dry wt. (g/plant)	0.32	0.43	0.40	0.02

CD=critical difference. Values are the mean of three replicates.

Rhizosphere Competence

The success of a microbial inoculant depends on its ability to survive and proliferate in the target habitat. The population density of rif-resistant mutant BIHB 783^{Rif} showed significant increase by the third week and remained significantly higher through six weeks than the initial count in pea rhizosphere (Fig. 5). The population build-up and recovery of the mutant from the non-rhizosphere was significantly lower than the rhizosphere population count. The total bacterial population throughout eight weeks was significantly higher in the rhizosphere of plants inoculated with the rif-resistant mutant than the bacterial population of uninoculated plants (Fig. 5). In the non-rhizosphere, the bacterial population of inoculated plants was statistically not different from uninoculated plants throughout the eight weeks. The total viable counts of fungi and actinomycetes in rhizospheres as well as non-rhizospheres showed a non-significant difference between uninoculated and inoculated plants (Fig. 5). A significant increase in root length, shoot length, and dry matter was also recorded on inoculation with both wild strain and mutant strain over uninoculated control (Table 2). The increase in plant growth by the mutant strain was not statistically different from the wild strain. The rapid colonization, sustainability of a substantial population in the rhizosphere, and dispersion of only a small population to the non-rhizosphere of the rifampicin-resistant mutant of *Rahnella* sp. strain BIHB 783 demonstrated high rhizosphere competence, which is an important attribute in the selection of isolates for developing plant growth-promoting formulations [3, 29]. The strain also appeared ecologically benign, as it promoted plant growth without significantly affecting the resident microbial population. The rhizosphere competence of bacterial strains is determined by their adaptability to the rhizosphere environment influenced by biotic and abiotic soil factors and response to root exudates [52].

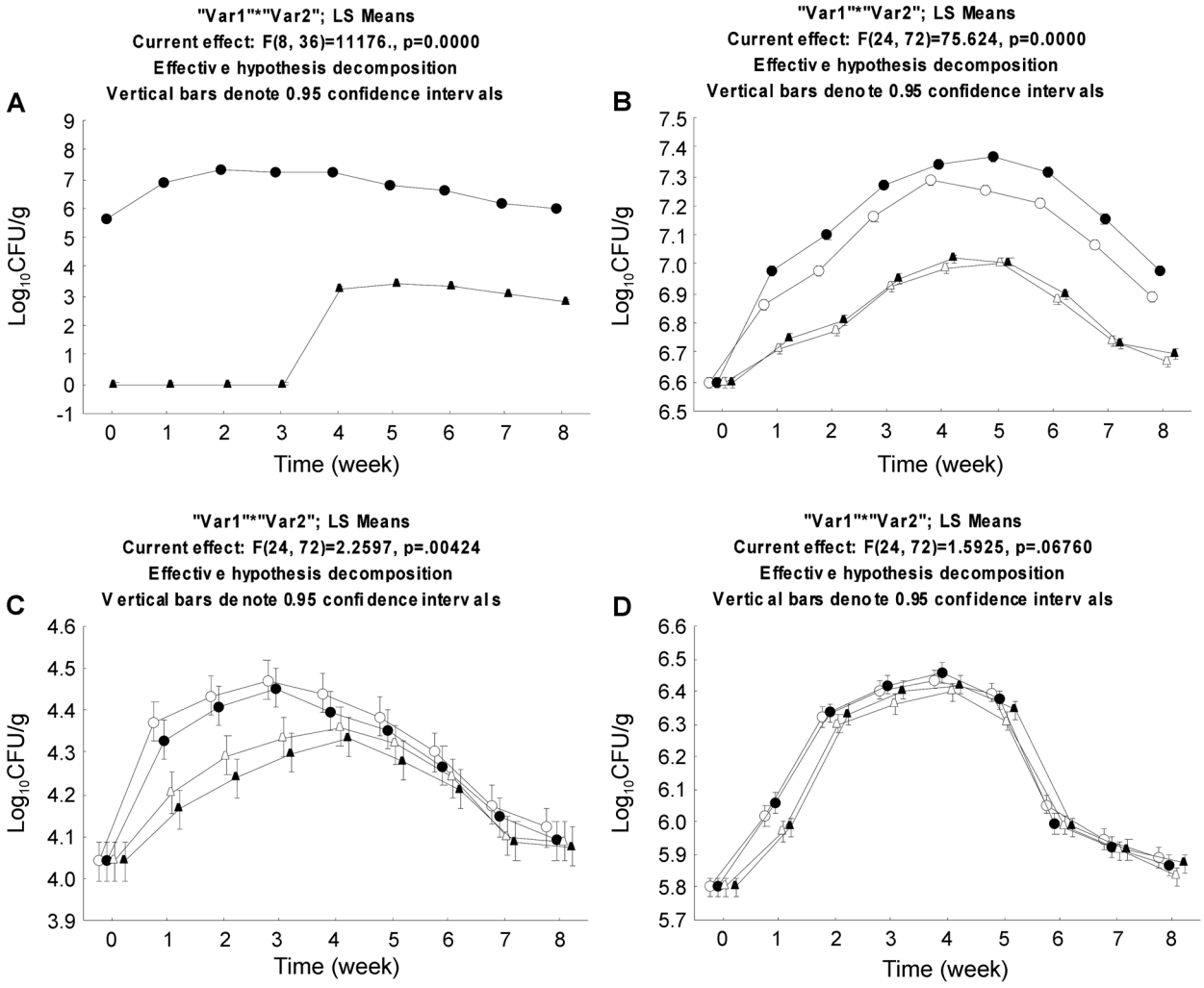

Fig. 5. Rhizosphere competence of *Rahnella* sp. BIHB 783 for pea in non-sterile soil under controlled environment: population density of BIHB 783^{Rif} in rhizosphere and non-rhizosphere (**A**); and influence of BIHB 783^{Rif} on rhizospheric and non-rhizospheric populations of bacteria (**B**), fungi (**C**), and actinomycetes (**D**).
○ Rhizosphere Control; ● Rhizosphere Inoculated; △ Non-Rhizosphere Control; ▲ Non-Rhizosphere Inoculated.

Influence on Plant Growth

A significant incremental influence on growth was observed on inoculation with *Rahnella* sp. BIHB 783 in the test plants grown in pots under controlled conditions (Table 3). The increment in root length, shoot length, and dry matter in inoculated plants over control was 31%, 44%, and 19% in maize, 23%, 40%, and 30% in pea, 20%, 32%, and 40% in chickpea and 33%, 25%, and 44% in barley, which showed a broad spectrum of plant growth-promoting activity by the test strain. Growth promotion in maize and

Table 3. Effect of *Rahnella* sp. strain BIHB 783 on the growth of test plants in sterilized vermiculite after 30 days in an Environment Control Chamber.

Growth parameter	*Zea mays* var. Girija		*Pisum sativum* var. Palam Priya		*Cicer arietinum* var. HPG 17		*Hordeum vulgare* var. Dolma	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
Root length (cm)	15.9	20.9* (31.4)	12.6	15.5* (23.0)	13.4	16.1* (20.1)	16.4	21.8* (32.9)
Shoot length (cm)	30.9	44.5* (44.0)	18.1	25.4* (40.3)	14.8	19.5* (31.8)	25.6	32.0* (25.0)
Dry wt. (g/plant)	0.32	0.38* (18.7)	0.20	0.27* (35.0)	0.1	0.14* (40.0)	0.09	0.13* (44.4)

Values are the mean of eight replicates. *Significantly different from control at $P<0.01$. Values in parentheses are the % increase over control.

Table 4. Effect of *Rahnella* sp. strain BIHB 783 on the growth of *Pisum sativum* var. Palam Priya after 110 days of sowing in microplots.

Location	Growth parameter	Control	Inoculated	CD at 5%
IHBT Chandpur Experimental Farms, Palampur	Shoot length (cm)	57.7	72.3 (25.3)	8.13
	Dry wt. (g/plant)	0.59	0.78 (32.2)	0.096
	Yield (pod fresh wt. kg/plot)	1.15	1.58 (37.4)	0.39
CSK HPKV Experimental Farms, Bajaura	Shoot length (cm)	58.5	76.8 (31.3)	9.58
	Dry wt. (g/plant)	0.57	0.81 (42.1)	0.15
	Yield (pod fresh wt. kg/plot)	1.23	1.74 (41.5)	0.42

CD=critical difference. Values are the mean of three replicates with nine plants each. Values in parentheses are the % increase over control.

Indian mustard by *Rahnella aquatilis* has been reported in pot cultures under controlled environment [11, 28]. The psychrotolerant strains of *Exiguobacterium acetylicum*, *Pantoea dispersa*, *Pseudomonas fragi*, *Pseudomonas* sp., and *Serratia marcescens* native to the North-western Indian Himalayas have also been reported for growth promotion in wheat in pot experiments [30, 43, 44, 45, 46]. The inoculum testing in pea also significantly enhanced growth and yield under the field conditions (Table 4). The increases in shoot length, dry weight, and yield were 25%, 32%, and 37% at the IHBT Experimental Farms, Palampur and 31%, 42%, and 41% at the CSK HPKV Experimental Farms, Bajaura. An increase of 15% in root length, 28% in dry matter, and 33% in yield has been reported in pea grown in microplots by *Acinetobacter rhizosphaerae* from the cold desert of the trans-Himalayas [18].

The cold tolerance, multiple plant growth-promoting attributes, high rhizosphere competence, and broad-spectrum plant growth-promoting activity revealed potential for *Rahnella* sp. BIHB 783 as a bioinoculant for improving agriculture productivity.

Acknowledgments

The Authors acknowledge the Director, Institute of Himalayan Bioresource Technology for providing necessary facilities and the Council of Scientific and Industrial Research, Govt. of India is also acknowledged for financial support under the CSIR Network Project "Exploitation of India's Rich Microbial Wealth" (NWP 006). The financial support as Senior Research Fellowship to Ms. Pratibha Vyas and Mr. Praveen Rahi by the Council of Scientific and Industrial Research, Govt. of India is also acknowledged.

REFERENCES

1. Anandham, R., P. Indira Gandhi, M. Madhaiyan, and T. M. Sa. 2008. Potential plant growth promoting traits and bioacidulation of rock phosphate by thiosulfate oxidizing bacteria isolated from crop plants. *J. Basic Microbiol.* **48:** 439–447.
2. Bakker, A. W. and B. Schippers. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth reduction. *Soil Biol. Biochem.* **19:** 452–458.
3. Behbahani, M. 2010. Investigation of biological behavior and colonization ability of Iranian indigenous phosphate solubilizing bacteria. *Scientia Horticult.* **124:** 393–399.
4. Bell, C. R., G. A. Dickie, and J. W. Y. F. Chan. 1995. Variable response of bacteria isolated from grapevine xylem to control grape crown gall disease in planta. *Am. J. Enol. Vitic.* **46:** 499–508.
5. Berge, O., T. Heulin, W. Achouak, C. Richard, R. Bally, and J. Balandreau. 1991. *Rahnella aquatilis*, a nitrogen-fixing enteric bacterium associated with the rhizosphere of wheat and maize. *Can. J. Microbiol.* **37:** 195–203.
6. Cappuccino, J. C. and N. Sherman. 1992. *Microbiology: A Laboratory Manual*, pp. 125–179. Benjamin/Cummings Pub. Co., New York.
7. Castric, P. A. 1975. Hydrogen cyanide, a secondary metabolite of *Pseudomonas aeruginosa*. *Can. J. Microbiol.* **21:** 613–618.
8. Chung, K. R., T. Shilts, U. Erturk, L. W. Timmer, and P. P. Uneg. 2003. Indole derivatives produced by the fungus *Colletotrichum acutatum* causing lime anthracnose and postbloom fruit drop of citrus. *FEMS Microbiol. Lett.* **226:** 23–30.
9. Dey, R., K. K. Pal, D. M. Bhatt, and S. M. Chauhan. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth promoting rhizobacteria. *Microbiol. Res.* **159:** 371–394.
10. Duponois, R., M. Kisa, and C. Plenchette. 2006. Phosphate solubilizing potential of the nematophagous fungus *Arthrobotrys oligospora*. *J. Plant Nutr. Soil Sci.* **169:** 280–282.
11. Egamberdiyeva, D., D. Juraeva, L. Gafurova, and G. Höflich. 2002. Promotion of plant growth of maize by plant growth promoting bacteria in different temperatures and soils. *In* E. van Santen (ed.). *Making Conservation Tillage Conventional: Building a Future on 25 Years of Research*. Special Report No. 1, Proceedings of the 25th Annual Southern Conservation Tillage Conference for Sustainable Agriculture, Auburn, AL. Alabama Agricultural Experiment and Auburn University, AL 36849, USA.
12. El-Hendawy, H. H., M. E. Osman, and N. M. Sorour. 2005. Biological control of bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* by *Rahnella aquatilis*. *Microbiol. Res.* **160:** 343–352.

13. Farajzadeh, D., N. Aliasgharzad, N. S. Bashir, and B. Yakhchali. 2010. Cloning and characterization of a plasmid-encoded ACC deaminase from an indigenous *Pseudomonas fluorescens* FY32. *Curr. Microbiol.* **61:** 37–43.
14. Fernández, L. A., P. Zalba, M. A. Gomez, and M. A. Sagardoy. 2007. Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. *Biol. Fert. Soils* **43:** 805–809.
15. Ge, S. M., L. Tao, and S. F. Chen. 2009. Expression and functional analysis of aminotransferase involved in indole-3-acetic acid biosynthesis in *Azospirillum brasilense* Yu62. *Biochemistry* **74:** 81–84.
16. Glick, B. R., Z. Cheng, J. Czarny, and J. Duan. 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant Pathol.* **119:** 329–339.
17. Gulati, A., P. Rahi, and P. Vyas. 2008. Characterization of phosphate-solubilizing fluorescent pseudomonads from rhizosphere of seabuckthorn growing in cold deserts of Himalayas. *Curr. Microbiol.* **56:** 73–79.
18. Gulati, A., P. Vyas, P. Rahi, and R. C. Kasana. 2009. Plant growth promoting and rhizosphere competent *Acinetobacter rhizosphaerae* strain BIHB 723 from the cold deserts of Himalayas. *Curr. Microbiol.* **58:** 371–377.
19. Huang, H., H. Luo, Y. Wang, D. Fu, N. Shao, G. Wang, P. Yang, and B. Yao. 2008. A novel phytase from *Yersinia rohdei* with high phytate hydrolysis activity under low pH and strong pepsin conditions. *Appl. Microbiol. Biotechnol.* **80:** 417–426.
20. Idriss, E. E., O. Makarewicz, A. Farouk, K. Rosner, R. Greiner, H. Bochow, T. Richter, and R. Borriss. 2002. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant growth-promoting effect. *Microbiology* **148:** 2097–2109.
21. Jacobson, B. C., J. J. Pasternak, and B. R. Glick. 1994. Partial purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the plant growth promoting rhizobacterium *Pseudomonas putida* GR 12-2. *Can. J. Microbiol.* **40:** 1019–1025.
22. Jackson, M. L. 1973. *Soil Chemical Analysis*. Prentice Hall, New Delhi, India.
23. Johri, J. K., S. Surange, and C. S. Nautiyal. 1999. Occurrence of salt, pH and temperature-tolerant phosphate-solubilizing bacteria in alkaline soils. *Curr. Microbiol.* **39:** 89–93.
24. Khan, A. A., G. Jilani, M. S. Akhtar, S. M. S. Naqvi, and M. Rasheed. 2009. Phosphorus solubilizing bacteria: Occurrence, mechanisms and their role in crop production. *J. Agric. Biol. Sci.* **1:** 48–58.
25. Kim, K. Y., D. Jordan, and H. B. Krishnan. 1997. *Rahnella aquatilis*, a bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. *FEMS Microbiol. Lett.* **153:** 273–277.
26. Krieg, N. R. and J. G. Holt. 1984. *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Williams and Willkins, Baltimore
27. Kuklinsky-Sobrat, J., W. L. Araujo, R. Mendes, I. O. Geraldi, A. A. Pizzirani-Kleiner, and J. L. Azevedo. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environ. Microbiol.* **6:** 1244–1251.
28. Kumar, K. V., S. Srivastava, N. Singh, and H. M. Behl. 2009. Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*. *J. Hazard. Mater.* **170:** 51–57.
29. Lugtenberg, B. J. J., L. Dekkers, and G. V. Bloemberg. 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annu. Rev. Phytopathol.* **39:** 461–490.
30. Mishra, P. K., S. Mishra, G. Selvakumar, S. C. Bisht, J. K. Bisht, S. Kundu, and H. S. Gupta. 2008. Characterization of a psychrotolerant plant growth promoting *Pseudomonas* sp. strain PGERs17 (MTCC 9000) isolated from North Western Indian Himalayas. *Ann. Microbiol.* **58:** 561–568.
31. Nautiyal, C. S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.* **170:** 265–270.
32. Negi, Y. K., S. K. Garg, and J. Kumar. 2005. Cold tolerant fluorescent *Pseudomonas* isolates from Garhwal Himalayas as potential plant growth promoting and biocontrol agents in pea. *Curr. Sci.* **89:** 2151–2156.
33. Park, K. H., C. Y. Lee, and H. J. Son. 2009. Mechanism of insoluble phosphate solubilization by *Pseudomonas fluorescens* RAF15 isolated from ginseng rhizosphere and its plant growth-promoting activities. *Lett. Appl. Microbiol.* **49:** 222–228.
34. Patel, D. K., G. Archana, and G. N. Kumar. 2008. Variation in the nature of organic acid secretion and mineral phosphate solubilization by *Citrobacter* sp. DHRSS in the presence of different sugars. *Curr. Microbiol.* **56:** 168–174.
35. Payne, S. M. 1994. Detection, isolation and characterization of siderophores. *Methods Enzymol*. **235:** 329–344.
36. Penrose, D. M. and B. R. Glick. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth promoting rhizobacteria. *Plant Physiol.* **118:** 10–15.
37. Poonguzhali, S., M. Madhaiyan, and T. Sa. 2008. Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and phosphorus utilization of plants. *J. Microbiol. Biotechnol.* **18:** 773–777.
38. Rodríguez, R., R. Fraga, T. Gonzalez, and Y. Bashan. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth promoting bacteria. *Plant Soil* **287:** 15–21.
39. Richardson, A. E. and P. A. Hadobas. 1997. Soil isolates of *Pseudomonas* spp. that utilize inositol phosphates. *Can. J. Microbiol.* **43:** 509–516.
40. Sarwar, M. and J. W. T. Frankenberger. 1994. Tryptophan dependent biosynthesis of auxins in soil. *Plant Soil* **160:** 97–104.
41. Sasser, M. 1990. Tracking a strain using the microbial identification system. Technical Note 102, MIS, Newark, DE.
42. Schwyn, B. and J. B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **160:** 45–56.
43. Selvakumar, G., M. Mohan, M. S. Kundu, A. D. Gupta, P. Joshi, S. Nazim, and H. S. Gupta. 2008. Cold tolerance and plant growth promotion potential of *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash (*Cucurbita pepo*). *Lett. Appl. Microbiol.* **46:** 171–175.
44. Selvakumar, G., S. Kundu, P. Joshi, S. Nazim, A. D. Gupta, P. K. Mishra, and H. S. Gupta. 2008. Characterization of a cold-tolerant plant growth-promoting bacterium *Pantoea dispersa* 1A isolated from a sub-alpine soil in the North Western Indian Himalayas. *World J. Microbiol. Biotechnol.* **24:** 955–960.
45. Selvakumar, G., S. Kundu, P. Joshi, A. D. Gupta, and H. S. Gupta. 2009. Growth promotion of wheat seedlings by *Exiguobacterium*

acetylicum 1P (MTCC 8707) a cold tolerant bacterial strain from the Uttarakhand Himalayas. *Indian J. Microbiol.* **50:** 50–56.

46. Selvakumar, G., P. Joshi, S. Nazim, P. K. Mishra, J. K. Bisht, and H. S. Gupta. 2009. Phosphate solubilization and growth promotion by *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium isolated from a high altitude Himalayan rhizosphere. *Biologia* **64:** 239–245.
47. Son, H. J., G. T. Park, M. S. Cha, and M. S. Heo. 2006. Solubilization of insoluble inorganic phosphates by a novel salt and pH-tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. *Biores. Technol.* **97:** 204–210.
48. Tsavkelova, E. A., T. A. Cherdyntseva, S. G. Botina, and A. I. Netrusov. 2007. Bacteria associated with orchid roots and microbial production of auxin. *Microbiol. Res.* **162:** 69–76.
49. Vargas, F. R. D. and G. W. O'Hara. 2006. Isolation and characterization of rhizosphere bacteria with potential for biological control of weeds in vineyards. *J. Appl. Microbiol.* **100:** 946–954.
50. Vyas, P. and A. Gulati. 2009. Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiol.* **9:** 174.
51. Vyas, P., P. Rahi, and A. Gulati. 2009. Stress tolerance and genetic variability of phosphate-solubilizing fluorescent *Pseudomonas* from the cold desert of the trans-Himalayas. *Microb. Ecol.* **58:** 425–434.
52. Wei, H. L. and L. Q. Zhang. 2006. Quorum-sensing system influences root colonization and biological control ability in *Pseudomonas fluorescens* 2P24. *Antonie van Leeuwenhoek* **89:** 267–280.
53. Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane. 1991. 16S Ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173:** 697–703.